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(54) Title: METHOD FOR DETECTION OF DRUG-INDUCED MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE

(57) Abstract

The present invention relates to a method for the rapid and reliable detection of drug-induced mutations in the reverse transcriptase gene allowing the simultaneous characterization of a range of codons involved in drug resistance using specific sets of probes optimized to function together in a reverse-hybridization assay. More particularly, the present invention relates to a method for determining the susceptibility to antiviral drugs of HIV strains present in a biological sample, comprising: (i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample; (ii) if need be amplifying the relevant part of the reverse transcriptase genes present in said sample with at least one suitable primer pair; (iii) hybridizing the polynucleic acids of step (i) or (ii) with at least two RT gene probes hybridizing specifically to one or more target sequences with said probes being applied to known locations on a solid support and with said probes being capable of simultaneously hybridizing to their respective target regions under appropriate hybridization and wash conditions allowing the detection of homologous targets, or said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T is replaced by U; (iv) detecting the hybrids formed in step (iii); (v) inferring the nucleotide sequence at the codons of interest and/or the amino acids of the codons of interest and/or antiviral drug resistance spectrum, and possibly the type of HIV isolates involved from the differential hybridization signal(s) obtained in step (iv).

Figure 1: Natural and drug-induced variability in the vicinity of codons 41, 50, 67-70, 70-75, 151, 181-184, 215 and 219 of the HIV RT gene.

Region I

38	39	40	41	42	43	44
TGT	ACA	GAA	ATG	GAA	AAG	GAA
		G	*T*	G	A	
			C		G	

Region II

47	48	49	50	51	52	53
ATT	TCA	AAA	ATT	GGG	CCT	GAA
		G	*G*			
			C			
			C			

Region III

65	66	67	68	69	70	71	72
AAA	AAA	GAC	AGT	ACT	AAA	TGG	AGA
G	G	*A*		A	*G*		
				A	G		
				GA			
				G			

Region IV

73	74	75	76	77
AAA	TTA	GTA	GAT	TTC
G	*G*	G	C	
		AC		

Region V

148	149	150	151	152	153	154
GTG	CTT	CCA	CAG	GGA	TGG	AAA
	C		*AT*			
	G	G	A			
			T			

Region VI

180	181	182	183	184	185	186	187
ATC	TAT	CAA	TAC	ATG	GAT	GAT	TTA
		G	T	*A*	C	G	GG
				G			G
	G			G			

Region VII

212	213	214	215	216
TGG	GGA	TTT	ACC	ACA
	G	C	T	
		A	*TA*	
		C	*TT*	

Region VIII

217	218	219	220
CCA	GAC	AAA	AAA
	T	G	G
		C	
		G	

Fig 2a

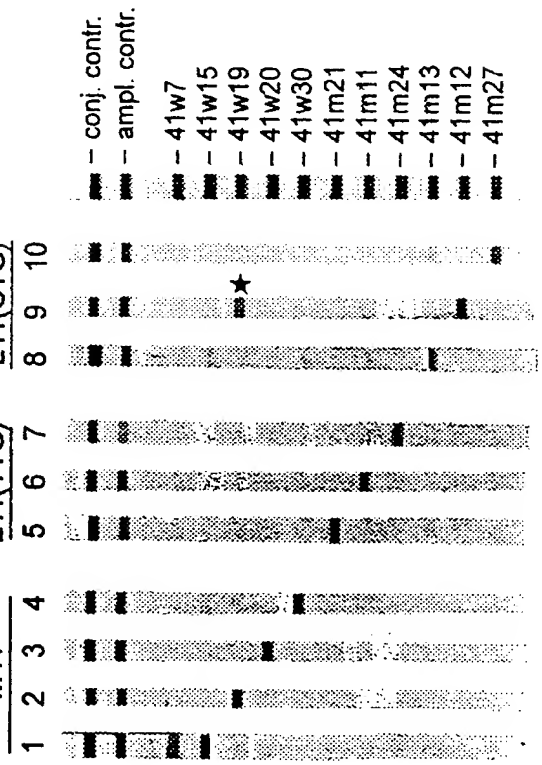


Fig 2b

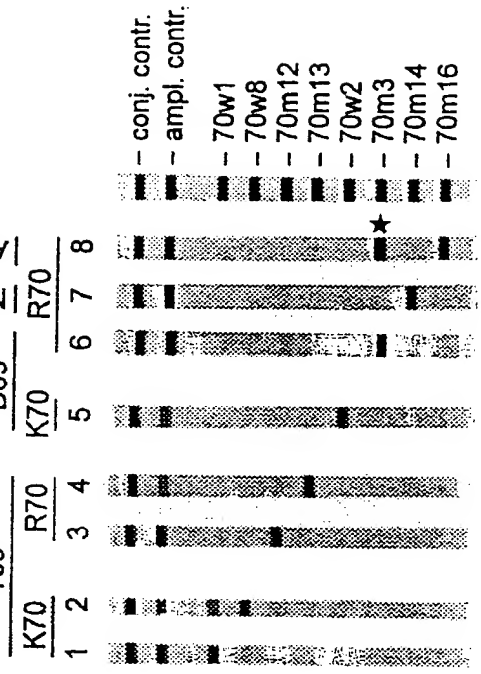


Fig 2c

Fig 2d

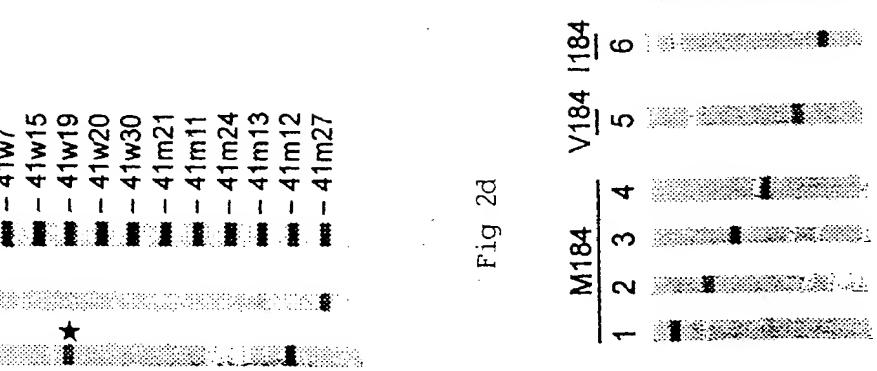


Fig 2f

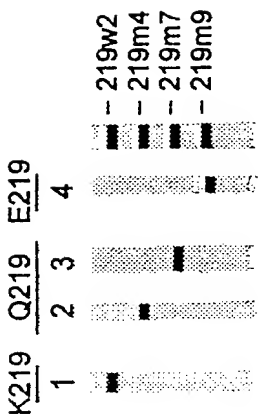


Fig 2e

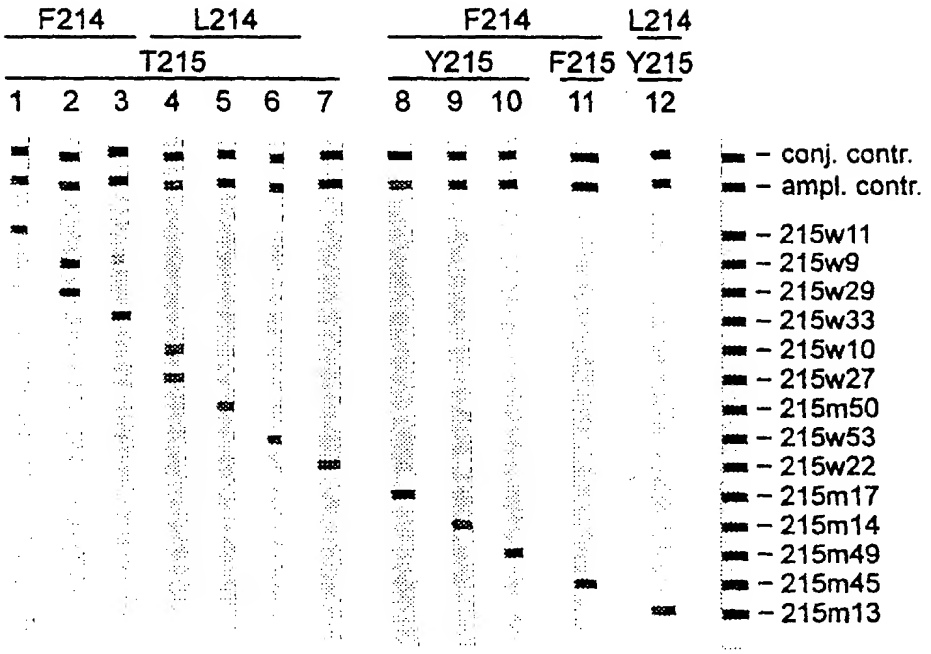
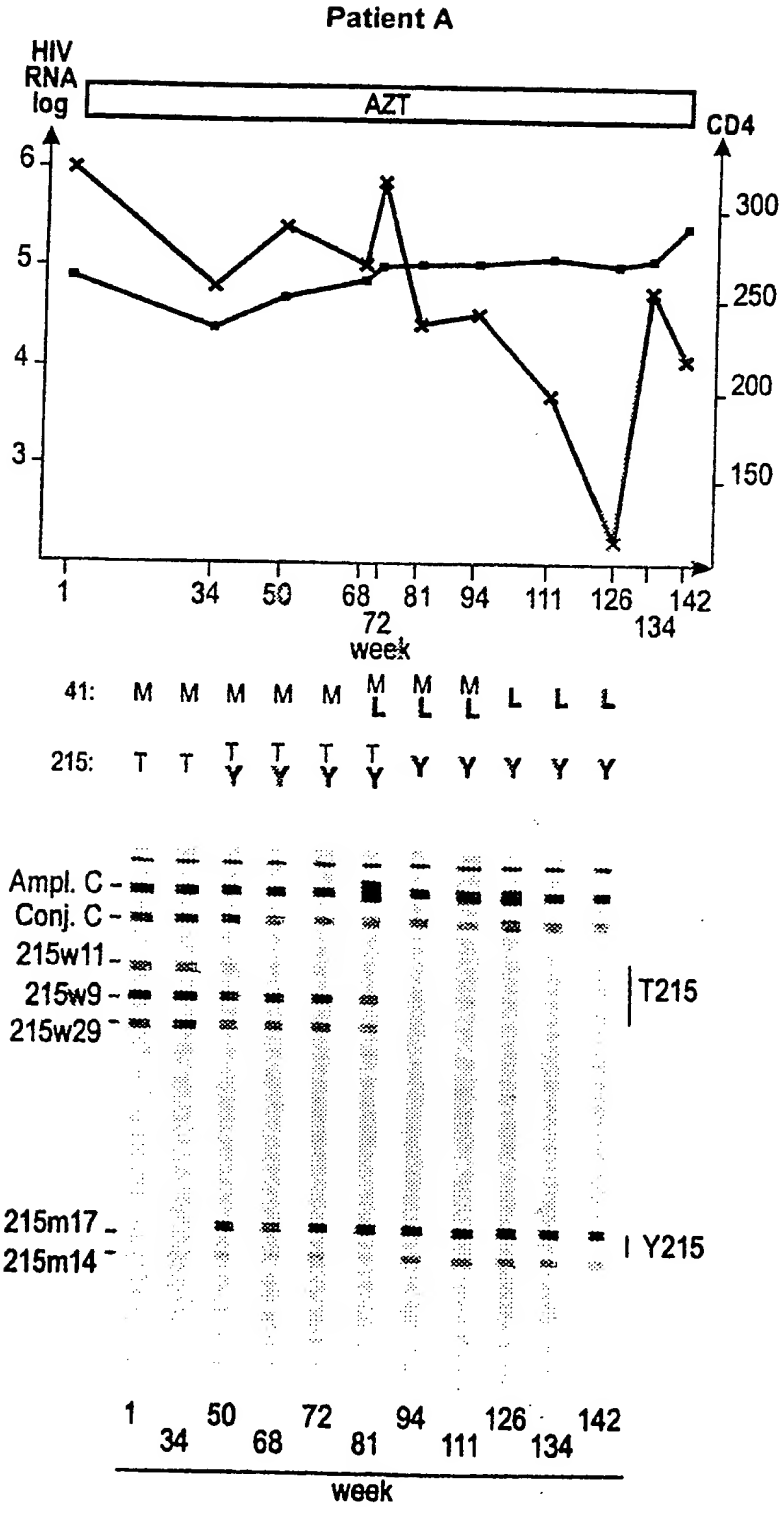
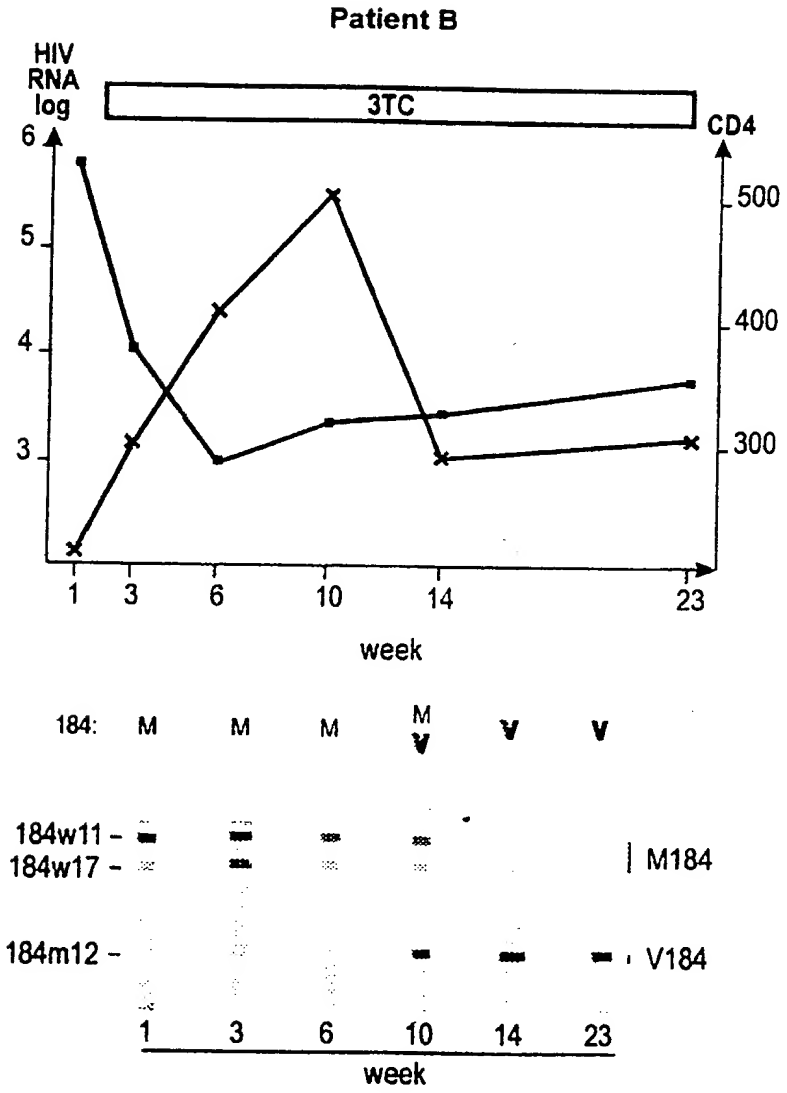

F214
L214
T215
F214
L214
Y215
F215
Y215
1 2 3 4 5 6 7 8 9 10 11 12
– conj. contr.
– ampl. contr.
– 215w11
– 215w9
– 215w29
– 215w33
– 215w10
– 215w27
– 215m50
– 215w53
– 215w22
– 215m17
– 215m14
– 215m49
– 215m45
– 215m13

Figure 3


Patient A
HIV RNA log
AZT
CD4
6
5
4
3
300
250
200
150
1
34
50
68
72
81
94
111
126
134
142
week
41: M M M M M M L M L M L L L
215: T T T Y T Y T Y T Y Y Y Y
Ampl. C
Conj. C
215w11
215w9
215w29
T215
215m17
215m14
I Y215
1
34
50
68
72
81
94
111
126
134
142
week


Patient B
HIV RNA log
3TC
CD4
6
5
4
3
500
400
300
1
3
6
10
14
23
week
184:
M
M
M
M
V
V
V
184w11 –
184w17 –
184m12 –
M184
V184
1
3
6
10
14
23
week

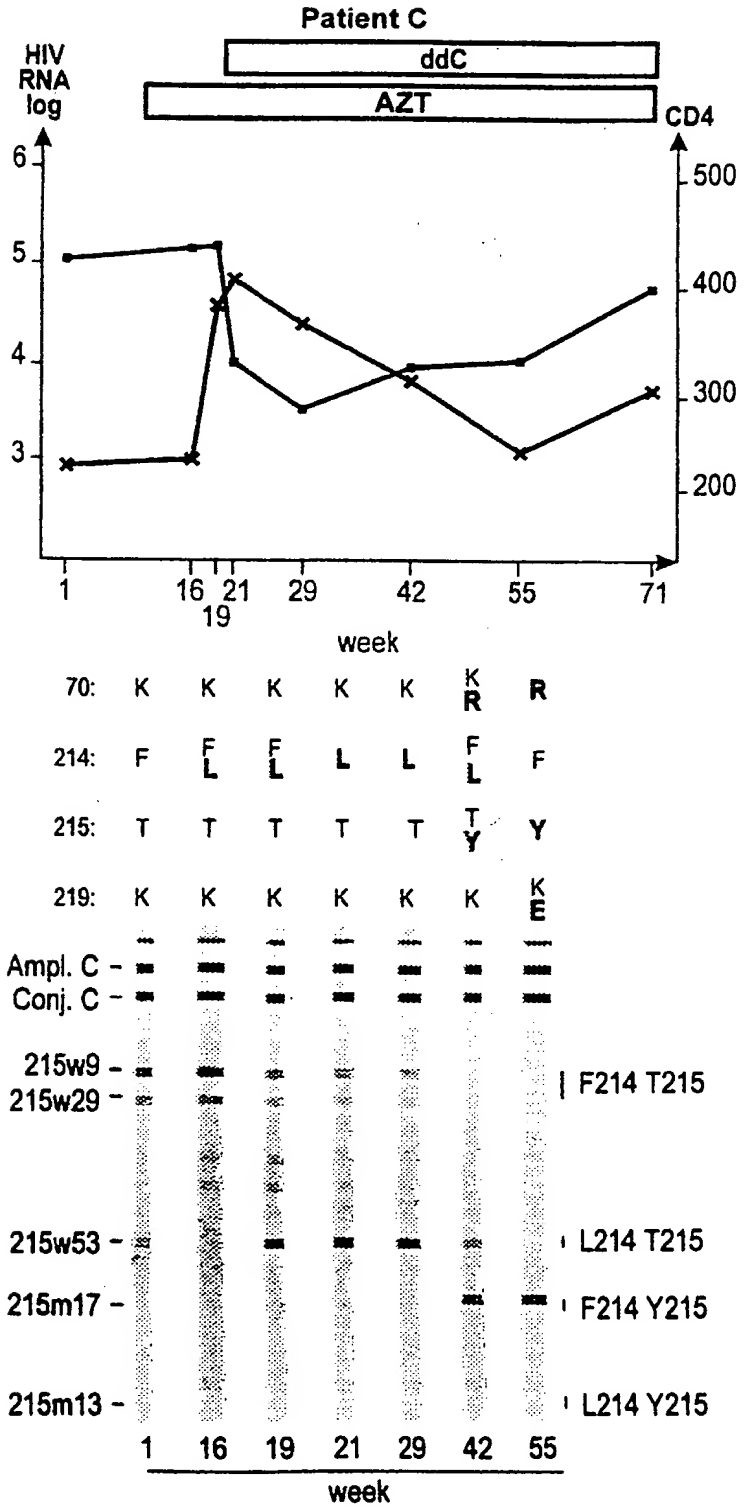
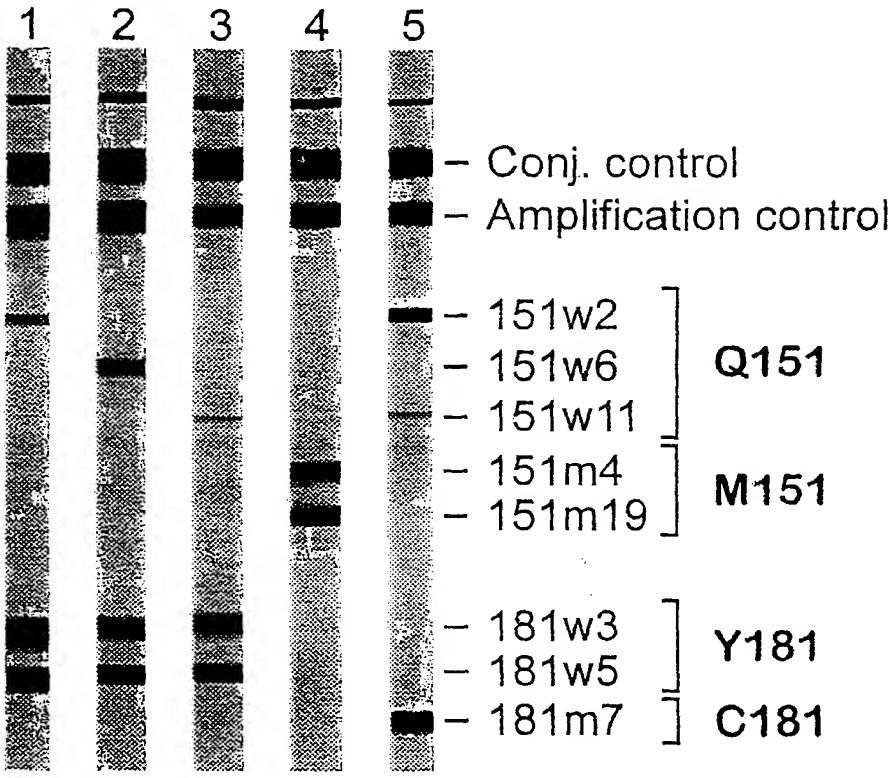

Patient C
HIV RNA log
ddC
AZT
CD4
6
5
4
3
500
400
300
200
1
16
19
21
29
42
55
71
week
70: K K K K K K R
214: F F L F L L F L F
215: T T T T T T Y Y
219: K K K K K K K E
Ampl. C
Conj. C
215w9
215w29
215w53
215m17
215m13
F214 T215
L214 T215
F214 Y215
L214 Y215
1 16 19 21 29 42 55
week

Figure 4


1
2
3
4
5
Conj. control
Amplification control
151w2
151w6
151w11
Q151
151m4
151m19
M151
181w3
181w5
Y181
181m7
C181